

Add the following new claim:

H7
--88. (new) An ex vivo cellular composition containing monocyte-derived antigen-presenting cells (MD-APCs) having been produced by differentiating blood monocytes in vitro, in the presence of lymphocytes, GM-CSF and at least one ligand having a receptor on the surface of monocytes, said cellular composition having, when compared with a cellular composition containing monocyte derived macrophages prepared in the presence of GM-CSF only, higher phagocytic properties of formalin fixed yeast and higher ability for stimulation of allogenic T lymphocytes.--

R E M A R K S

This application has been amended in a manner that is believed to place it in condition for allowance at the time of the next Official Action.

Claims 44, 46-47, 49-51, 53-55 and 60-61 have been amended to more patentably point out and distinctly claim the present invention. Claims 45 and 58 have been canceled. New claim 88 has been added.

The claimed invention has been amended to recite a monocyte derived antigen presenting cell (MD-APC). These MD-APCs are produced by differentiating blood monocytes *in vitro*, in the presence of lymphocytes, GM-CSF and at least one ligand having a receptor on the surface of monocytes, the MD-APCs having, when compared with monocytes derived macrophages prepared in the

presence of GM-CSF only, higher phagocytic properties of formulin fixed yeast and the higher ability for stimulation of alogenic T lymphocytes. Support for the recitation that the phagocytosis capacity may be evaluated by an uptake of formalin fixed yeast may be found at page 3, lines 20-30. The recitation of comparing the MD-APCs monocytes derived macrophages prepared in the presence of GM-CSF only may be found at page 14, line 16 to page 15, line 5. As to the recitation that the claimed MD-APCs exhibit higher phagocytic properties of formalin fixed yeast and higher ability for stimulation of alogenic T lymphocytes, the Examiner's attention is respectfully directed to page 9, lines 24-26 and page 9, lines 32-33. Thus, it is respectfully submitted that no new matter has been added to the present application.

In the outstanding Official Action, claims 44-47, 49-51, 53-55, 58 and 60-61 were rejected under 35 USC §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In light of the present amendment, this rejection is respectfully traversed.

Claims 45 and 55 were rejected for containing the term "relative to standard macrophages". Applicants respectfully submit that this term would be definite to one of ordinary skill in the art. Moreover, Applicants note that the term "standard macrophages" may be found in United States patents 5,662,899,

6,001,351 and 6,051,432. Nevertheless, in the interest of advancing prosecution, the claims have been amended so that this term is no longer recited. Thus, it is respectfully submitted that claims 44 and 55 are definite to one of ordinary skill in the art.

Claims 45 and 58 were rejected for allegedly being indefinite for containing the phrase "a high rate of phagocytic uptake". As claims 45 and 58 have been canceled, Applicants respectfully submit that the rejection of claims 45 and 58 have been rendered moot.

Claims 50 and 54 were rejected for containing the term "cultured". The Examiner thoughtfully suggested that the term "culture" would be more appropriate. In accordance with the Examiner's suggestion, claims 50 and 54 have been amended so that the term "cultured" has been deleted and the term "culture" has been inserted.

Claim 60 was rejected for being dependent on a canceled claim. Claim 60 has been amended so that it is now dependent on claim 55.

Thus, in light of the present amendment, it is respectfully submitted that claims 44, 46-47, 49-51, 53-55 and 61 are definite to one of ordinary skill in the art.

In the outstanding Official Action, claims 44-47, 49-51, 53-55, 58 and 61 were rejected under 35 USC §112, first paragraph, as allegedly containing new matter.

The outstanding Official Action alleged that the recitations of "mature dendritic cells" and "said MD-APCs having been produced by differentiating blood monocytes *in vitro*, in the presence of ligands enhancing the capacity of said MD-APCs for MHC-1 antigen presentation relative to standard macrophages" constituted new matter.

As noted above, in the interest of advancing prosecution, the claims have been amended so that these phrases are no longer recited. Thus, Applicants respectfully request that the new matter rejection be withdrawn.

Claims 44-47, 49, 51, 53-55, 58 and 60-61 were rejected under 35 USC §112, first paragraph, as allegedly being based on the insufficient written description and a non-enabling disclosure.

Applicants respectfully submit that the present specification discloses to one of ordinary skill in the art how to make the claimed MD-APCs and that the claimed MD-APCs exist as a single, specific, cell type. The claimed MD-APCs may be obtained according to the process of the present invention. The Examiner's attention is respectfully directed to page 5, lines 37 to page 8, line 9. Applicants respectfully submit that it would be clear to one of ordinary skill in the art that the present invention describes a process for preparing the claimed MD-APCs.

The process comprises isolating PBMCs from a healthy donor or a patient by apheresis; possibly isolating mononuclear

cells from red blood cells and granulocytes; possibly isolating mononuclear cells from other PBMCs; culturing the PBMCs or mononuclear cells, in a medium containing in combination or not with GM-CSF, at least one ligand of mononuclear cells; and possibly separating a monocyte derived APCs from monocytes.

The cells may be characterized by their phenotypic and functional characteristics. In particular, the comparative phenotypic and functional characterization of the claimed MD-APCs in monocyte-derived macrophages (MAK) are presented in publications from BOYER et al., (EXP. Hematol. 1999, 27, 751-761) and CHAPEROT et al. (Leukemia, 2000, 14, 1667-1677). These publications are submitted in accordance with the present amendment.

Upon reviewing BOYER et al. and CHAPEROT et al., it is believed to be apparent that the claimed MD-APCs indeed exist. For example, as noted above, the present specification describes a method for determining the cellular phagocytic properties for the uptake of formulin fixed yeast (see present specification page 3, lines 22-28). The results of the uptake of the yeast by the claimed MD-APCs is also demonstrated by BOYER et al. BOYER et al. compared the ability of macrophages and the claimed MD-APCs ability to capture and phagocytose yeast (Figures 2, 3 and 4). In contrast to the macrophages, a large number of yeast were found to be phagocytosed by the claimed MD-APCs. No single

macrophage is able to phagocytose more than 15 yeast whereas about 40% of the MD-APCs did (Figure 3).

Moreover, the present specification describes a method for determining the stimulating properties of the claimed MD-APCs on allogenic T cells (see present specification page 4, lines 20-27). In fact, Applicants note that Figures 1a and 1b show results showing the superior proliferation stimulators of the claimed MD-APCs compared to monocyte derived macrophages, obtained in the presence of GM-CSF only.

In CHAPEROT et al., monocytes from the same patient were used for producing, for one part, the claimed MD-APCs and, for the other part, macrophages. As to the results, Figure 2 shows a greater ability of MD-APCs to stimulate T cell proliferation than monocytes and macrophages, the level of response being 3 to 20 fold higher at 0.5/1 APC/T lymphocyte ratio.

Thus, it is respectfully submitted that the present specification clearly discloses a how to make the claimed MD-APCs.

The outstanding Official Action cites United States patent No. 5,851,756 as supporting the contention that the present specification does not adequately disclose to one of ordinary skill in the art that the MD-APCs exist as a single cell type. The Official Action contends that it is well known to one of ordinary skill in the art that the culturing of monocytes with

GM-CSF for a time of several days will result in the generation of dendritic cells.

However, this patent describes a method of producing a population of dendritic cell precursors from proliferating cell cultures. The method comprises providing a tissue source comprising dendritic precursors, such as blood and bone marrow, then treating the tissue source to increase the proportion of the dendritic cell precursors, prior to culture. A treatment may be culturing the tissue source on a substrate so as to select non-inherent cells (see column 5, lines 28-29), or removing and killing undesirable non-dendritic cell precursors (see column 5, lines 36-42). The undesirable cells are T and B cells as well as monocytes (see column 12, line 47; column 15, lines 9-11; and Figure 1). Thus, Applicants respectfully submit that the method set forth in United States patent application 5,851,756 fails to describe or suggest that the culturing of monocytes with the GM-CSF results in a generation of dendritic cells.

The outstanding Official Action also contends that the specification comprises a number of confusing disclosures. However, it is respectfully submitted that Tables 1, 2 and 3 in the present specification establish that the claimed MD-APCs are obtained in the culture system of the present invention. Moreover, it is believed that the Tables show that the MD-APCs are distinct from dendritic cells (DC), since DC have been described as FC (CD64) receptor negative, non-adherent and non-

phagocytic cells, possessing only a small number of lysosomes. In contrast, the claimed MD-APCs show a high adherence capacity, show an important phagocytic and processing activity, and express high levels of HLA-DR membrane antigens in a low level of CD1a and CD1c, which is strongly expressed by dendritic cells.

As to distinguishing the claimed MD-APCs from macrophages, Applicants note that a self preparation containing the claimed MD-APCs would function differently from a cell preparation of macrophages and that the cell preparation would have the ability to phagocytose tumor cells or particles such as yeast and have the ability to stimulate allogenic autologous T lymphocytes.

Table 1 indicates the yield of living cells in a culture related to the number of cells at the beginning of the culture (days 4, 7 and 11). This yield diminishes with the duration of the culture from 71% to 19% and from 78-31%, in the presence or not of GM-CSF. In Table 2, the absence of CD83⁺ cells clearly shows the absence of "classical" dendritic cells. Thus, one of ordinary skill in the art would clearly conclude that the lymphocytes stimulation is due to the MD-APCs according to the present invention, differing from macrophages.

As to Table 3, the MD-APCs are tested for phagocytic activity using formalin fixed yeast. After three hours of incubation and standing, the intracellular particles are quantified by microscopic analysis. The microscopic analysis

reveals that the MD-APCs generated in the presence of histamine/cimetidine exhibit.

In fact, Table 4 provides a direct comparison of the MD-APCs of the present invention and the dendritic cells. Table 5 gathers a complete phenotypic characterization of MD-APCs recovered after six days of culture according to the method of the present invention. The analysis is conducted by flow cytometry. Applicants note that Table 5 has been amended in the outstanding Official Action, the Examiner alleged that Table 5 was indecipherable as it was unclear what a numerical percentage with a comma in the middle indicated. Applicants respectfully submit that the numerical percentages with a comma were a result of the translation of the present application. As such, Table 5 has been amended so that these commas have been removed and replaced with an appropriate period.

Figures 1a and 1b compare allogenic T cell proliferation induced by MD-APCs or by macrophages. The proliferation is assessed by a colorimetric method, based on the detection of the hydrolysis of tetrazolium salts WST-1 (slightly red) to Formazan (dark red) (see page 3 of the specification) which is represented on the Y axis, as indicated by an optical density (OD) from 450 to 490 nm characteristic of red color. The optical density is plotted against APC/lymphocytes ratios.

Figure 1a represents the allogenic T cell proliferation induced by monocyte-derived cells. APCs were prepared according

to different conditions. The lighter grey bars correspond to cells incubated in the presence of histamine 10^{-4} M and cimetidine 10^{-6} M (condition 1), the clear (intermediary) dotted bars correspond to cells incubated in the presence of GM-CSF (500 U/ml), the darker grey bars correspond to cells incubated in the presence of histamine 10^{-4} M, cimetidine 10^{-6} M and GM-CSF (500 U/ml) (condition 2). Cells prepared in presence of GM-CSF only correspond to macrophages.

Conditions 1 and 2 lead to the production of monocyte-derived cells according to the invention. The results show that, for all the monocyte derived cells/lymphocytes ratios tested, MD-APCs according to the present invention, corresponding to cells prepared with histamine and cimetidine, or histamine + cimetidine + GM-CSF are more potent for inducing allogenic T cells proliferation than standard macrophages, obtained in the presence of GM-CSF only.

Figure 1b represents the stimulation of the proliferation of allogenic T lymphocytes by different MD-APCs of the invention compared to standard macrophages, obtained by incubating monocytes in the presence of GM-CSF (500 ng/ml) only.

In Figure 1b, the optical density (450-690 nm) has been plotted against the ratio of APCs/LT (see page 15, lines 1-2).

Thus, MD-APCs according to the method were obtained by using GM-CSF (500 U/ml) and IL-13 (50 ng/ml), whereas macrophages were obtained by using only GM-CSF (500 UI/ml) in the incubation

medium. Results show that MD-APCs of the present invention are more potent as inducers of T cells proliferation than macrophages. The presence of IL-13 in the incubation medium leads to the obtention of cells that present a capacity of inducing allogenic T lymphocytes proliferation superior to that of macrophages, obtained by using GM-CSF only.

The outstanding Official Action also alleges that the present specification does not support the phrases "MD-APCs which have a higher phagocytic capacity than mature dendritic cells", "mature dendritic cells", "MD-APCs having a greater capability of stimulating proliferation of allogenic lymphocytes relative to standard macrophages" and "in the presence of ligands enhancing the capacity of said MD-APCs or MHC-1 antigen presentation relative to standard macrophages". However, as these recitations are no longer recited in the claims, it is respectfully submitted that the contentions pertaining to these phrases have been obviated.

Thus, it is respectfully submitted that claims 44, 46-47, 49-51, 53-55, 60 and 61 are supported by a sufficient written description and enabled by the present disclosure.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance, with claims 44, 46-47, 49-51, 53-55, 60 and 61, as presented. Allowance and passage to issue on that basis are accordingly respectfully requested.

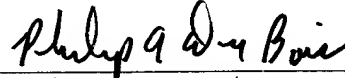
CHOKRI et al. S.N. 09/194,053

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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By



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Page 20, Table 5 has been replaced as follows:

Table 5

It gathers a complete phenotypic characterization of MD-APCs recovered after 6 days of culture according to the invention (analysis by flow cytometry).

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Phenotype	% Cells	Mean fluo Intensity
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CD45	[97,6] <u>97.6</u>	172
CD14	[6,8] <u>6.8</u>	
CD3	13	
CD19	15	
CD56	[3,8] <u>3.8</u>	
CD4-PE	95	
CD25	[1,7] <u>1.7</u>	
CD45RO	99	

CD16	[1,8] <u>1.8</u>	31
CD32	63	163
CD64	4	12

CD1a	31	216
CD1c	58	505
CD83	9	18

HLA-DR	99	266
HLA-I	[99,6] <u>99.6</u>	582
CD40	98	991
CD80	78	64
CD86	99	744

IgG1-FITC	[6,7] <u>6.7</u>	11
IgG1-PE	20	(16) 55
IgG1-Cy5	19	(29) 50
IgG2a-FITC	[5,3] <u>5.3</u>	19
IgG1 i	[1,6] <u>1.6</u>	75
IgG2a i	[3,8] <u>3.8</u>	21
IgG2b i	[3,2] <u>3.2</u>	15

Claim 44 has been amended as follows:

--44. (four times amended) Monocyte-derived antigen-presenting cells (MD-APCs) [which have a higher phagocytic capacity than mature dendritic cells and which have capacity for MHC class I (MHC-I) and MHC class II (MHC-II) antigen presentation,

said MD-APCs] having been produced by differentiating blood monocytes in vitro, in the presence [of ligands enhancing the capacity of said MD-APCs for MHC-I antigen presentation relative to standard macrophages,

said MD-APCs having a greater capability of stimulating proliferation of allogenic lymphocytes relative to standard macrophages] of lymphocytes, GM-CSF and at least one ligand having, a receptor on the surface of monocytes, said MD-APCs, having when compared with monocyte derived macrophages prepared in the present of GM-CSF only, higher phagocytic properties of formalin fixed yeast and higher ability for stimulation of allogenic T lymphocytes.--

Claim 50 has been amended as follows:

--50. (twice amended) The monocyte-derived antigen-presenting cells of claim 44, wherein said monocyte-derived antigen-presenting cells present adherent properties as determined by MD-APCs [cultured] culture for 2 hours in culture medium of one of I.M.D.M. and R.P.M.I. on plastic flasks and the percentage of adherent cells is quantified by microscopic analysis.--

Claim 54 has been amended as follows:

--54. (twice amended) The monocyte-derived antigen-presenting cells of claim 47, wherein said monocyte-derived antigen-presenting cells present adherent properties as determined by MD-APCs [cultured] culture for 2 hours in a culture medium of one of I.M.D.M. and R.P.M.I.--

Claim 55 has been amended as follows:

--55. (thrice amended) Monocyte-derived antigen-presenting cells (MD-APCs) which present the following properties:

(c) the presence on the MD-APC cell surface of surface antigens CD80 and CD86; and

(d) the presence on the MD-APC cell surface of surface antigen CD14, [and

(e) a higher phagocytic capacity than mature dendritic cells,]

said MD-APCs [having] have been produced by differentiating blood monocytes in vitro, in the presence [of

ligands enhancing the capacity of said MD-APCs for MHC-I antigen presentation relative to standard macrophages,

said MD-APCs having a greater capability of stimulating proliferation of allogenic lymphocytes relative to standard macrophages] of lymphocytes, GM-CSF and at least one ligand having a receptor on the surface of monocytes, said MD-APCs having, when compared with monocyte derived macrophages prepared in the presence of GM-CSF only, higher phagocytic properties of formalin fixed yeast and higher ability for stimulation of allogenic T lymphocytes.--

Claim 60 has been amended as follows:

--60. (amended) The monocyte-derived antigen-presenting cells of claim [59] 55, wherein said monocyte-derived antigen-presenting cells are substantially devoid of the surface antigens CD1a and CD1c.--